

## PARAOXONASE 1 AND CARDIOVASCULAR RISK IN NEPHROTIC SYNDROME PATIENTS

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### ABSTRACT

**Background:** Abnormal lipoprotein metabolism is a common characteristic of nephrotic syndrome. But this dyslipidemia cannot fully explain the increased incidence of atherosclerosis in this population. HDL associated enzyme, paraoxonase 1 (PON1), prevents the formation of oxidized LDL, which is an important culprit in the development of cardiovascular disease and therefore reduced PON1 is found to be an independent risk factor for atherosclerosis.

**Objective:** The primary intention of this study is to analyze the PON1 levels in nephrotic syndrome along with lipid profile and atherogenic index, thus trying to provide a clearer picture of cardiovascular risk in nephrotic syndrome patients.

**Method:** 40 newly diagnosed nephrotic syndrome patients were included in the study. Equal number of age and sex matched healthy controls were also selected. Basal and salt stimulated activity of PON1 was measured along with lipid profile and atherogenic index.

**Results:** There was a significant decrease of PON1 activity in nephrotic syndrome patients compared to the healthy controls. Total cholesterol, LDL, triglycerides, VLDL and Atherogenic Index were high and HDL cholesterol level was low in nephrotic syndrome patients. Moreover a significant negative correlation was observed between PON1 and Atherogenic index and a positive correlation between PON1 and HDL-cholesterol in both cases and controls.

**Conclusions:** Therefore the study reveals an increased risk of cardiovascular disease in nephrotic syndrome patients. Hence measuring PON1 and Atherogenic index during the initial stages of nephrotic syndrome may help in predicting the extent of the associated cardiovascular risk.

**KEYWORDS:** Atherogenic Index, Cardiovascular Risk, Lipid Profile, Nephrotic Syndrome, Paraoxonase 1

### INTRODUCTION

Nephrotic Syndrome is a common chronic kidney disorder being found in all age groups (1). It is a constellation of clinical symptoms characterized by proteinuria of  $>3.5\text{g}/1.7\text{m}^2/24\text{ hr}$ , hypoalbuminemia, edema, hyperlipidemia, lipiduria and hypercoagulability (2). The prevalence of nephrotic syndrome is difficult to establish in adults, since it varies depending

on the etiology and the age of onset. In children it is about 16 cases per 1,00,000(3). Thromboembolism is one of the most significant life threatening complications of nephrotic syndrome and about 58% of the mortality in nephrotic syndrome is due to cardiovascular disease (5). Incidence of thromboembolism in nephrotic syndrome patients is as high as 25% in adults and 3% in children (6). This has been mainly attributed to be induced by dyslipidemia and hypercoagulability (7).

Disturbances in lipid metabolism are common in nephrotic syndrome. These patients have remarkable abnormalities in lipoprotein metabolism, and the magnitude of the changes correlates with the severity of the disease(4). Increased total cholesterol, triglycerides, LDL, VLDL and decreased HDL is the picture of lipid profile in nephrotic syndrome. But this dyslipidemia cannot fully explain the increased incidence of atherosclerosis in this population. Oxidized LDL levels are elevated in nephrotic syndrome, which is an independent risk factor for cardiovascular disease. LDL oxidation occurs due to increased oxidative stress which can be a consequence of hypoalbuminemia (8). Thus oxidant-antioxidant imbalance seen in nephrotic syndrome also adds to the cardiovascular risk.

Antioxidant enzymes and other antioxidants play a major role in decreasing the basement membrane injury caused by reactive oxygen species (11). Paraoxonase 1 is a calcium dependent esterase which is synthesized in the liver (12). It is a lipophilic antioxidant component of HDL cholesterol and has been shown to reduce the susceptibility of LDL to lipid peroxidation (13) probably by hydrolyzing specific lipid peroxides, thereby decreasing the oxidized LDL levels. Thus reduced PON1 is also an independent risk factor for atherosclerosis. Soyoral et al studied the serum PON1 activity and oxidative stress in patients with nephrotic syndrome where they found reduced PON1 activity and increased oxidative stress in nephrotic syndrome (14). PON1 activity in children with nephrotic syndrome has been evaluated by Mohammed Hashemi et al (11).

## OBJECTIVE

The primary intention of this study is to compare the PON1 levels in nephrotic syndrome patients and healthy controls, and also to correlate the PON1 levels with the severity of cardiovascular risk in nephrotic syndrome cases. Whether PON1 could be used to find out the extent of cardiovascular risk in nephrotic syndrome patients?

## MATERIALS AND METHODS

This case control study was conducted in the Department of Biochemistry, Kasturba Medical College, Mangalore with approval of Institutional Ethical Committee. A total of 80 subjects between 5 to 55 years of age were included. The subjects were further divided into two groups

**Group 1:** 40 healthy controls

**Group 2:** 40 patients with Nephrotic syndrome.

The patients were all admitted to KMC hospital Mangalore/Manipal or Wenlock District Hospital, Mangalore in the one year period from February 2013 to February 2014. They were newly diagnosed nephrotic syndrome cases by biopsy evidence and other clinical investigations like proteinuria of  $>3.5\text{g}/1.73\text{ m}^2/24\text{hr}$ , hypoalbuminemia, edema, hyperlipidemia, lipiduria and hypercoagulability. Written informed consent was obtained from all subjects. Patient who had chronic renal failure, hemodialysis, cardiac diseases, liver diseases with nephritis, cancer, other systemic diseases such as lupus nephritis, smokers and alcoholics were excluded from this study. Healthy control subjects were matched for age and

sex and not suffering from the conditions which may alter oxidative status. Nephrotic syndrome patients and control subjects were not on any supplementation like vitamins or minerals.

About 4ml of venous blood was collected after 8 hours of overnight fasting, in a sterile plain vacutainer and instantly serum was separated. The samples were stored at -80 °C until analysis. Total Cholesterol, HDL-cholesterol and Triglycerides were estimated by commercially available kits, in semi-auto analyzer. LDLC and VLDLC were calculated using Friedwalds formula.

**PON1 Estimation:** This enzyme was estimated spectrophotometrically using 5.5 mM 4-nitrophenylacetate as the substrate in 20 mM Tris–HCl buffer at a pH of 8.0. The increase in absorbance due to the formation of the yellow 4-nitrophenol was monitored at 412 nm for 3 mins. For each sample basal PON activity as well as salt- stimulated PON activity was determined as described below [15].

**Basal PON (BPON):** This was estimated by using the Tris–HCl buffer containing only 1 mM calcium chloride. *Salt-stimulated PON (SPON):* This was estimated by using the same Tris–HCl buffer which however contained 1 mM calcium chloride as well as 1 M NaCl.

PON activity was calculated after making corrections for non-enzymatic hydrolysis. An extinction coefficient of 18,000/M/cm was used to calculate the PON1 activity. 4-nitrophenol acetate was procured from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used for the assay of PON were of analytical grade.

**Statistical Analysis:** All values are expressed as mean  $\pm$  SD. Statistical analysis was done using SPSS 16. Biochemical parameters in cases and controls were compared using paired 't' test. Pearson correlation was used to correlate PON1 with HDL cholesterol and Atherogenic index. P value <0.05 was considered statistically significant. ROC curve was used to compare the sensitivity and specificity of BPON and SPON in determining the cardiovascular risk in nephrotic syndrome patients.

## RESULTS

The results of paraoxonase activities, lipid profile and Atherogenic index are mentioned in Table 1. The box and whisker plot in Figure 1 shows that the BPON values ranged from 38.89 to 104.44 IU/ml in cases with a median value of 67.78 IU/ml, while the SPON values ranged from 44.44 to 112.22 IU/ml with a median of 75.56 IU/ml. In control group BPON activity ranged from 51.11 to 105.56 IU/ml with a median of 82.78 IU/ml and SPON activity ranged from 62.22 to 122.22 IU/ml with a median of 89.44 IU/ml.

Figure 2 Shows the correlation between paraoxonase 1 and Atherogenic index in cases and Figure 3 shows the correlation between PON1 levels and HDL cholesterol in cases. There was a strong negative correlation between PON1 and Atherogenic index in cases (Pearson correlation coefficient,  $r=0.798$ ) and an intermediate correlation in controls ( $r=0.573$ ). Also there was an intermediate positive correlation between PON1 and HDL cholesterol in cases ( $r=0.479$ ) and no correlation in controls ( $r=0.270$ ).

ROC curve in Figure 4, assess the efficiency of SPON and BPON in determining the cardiovascular risk in nephrotic syndrome subjects. Area under the curve for BPON was 0.770 while that for SPON was 0.758 and the difference showed a p value <0.001.

## DISCUSSIONS

There are only very few studies which measured the paraoxonase activity in nephrotic syndrome patients. Paraoxonase 1 is an HDL associated antioxidant enzyme and its reduced level is an independent risk factor of cardiovascular disease. As mentioned in the introduction, cardiovascular disease prevalence in nephrotic syndrome is very high. Therefore it is advisable to measure the cardiac risk factors in early stages of nephrotic syndrome to prevent the early onset of cardiovascular diseases. This study evaluates the paraoxonase activity in nephrotic syndrome patients. The box and whisker plot in Figure 1 shows that there is significant decrease in PON1 levels in nephrotic syndrome cases when compared to that of the controls. Soyoral et al in 2011 found out that oxidative stress increased while serum PON1 activity was decreased in patients with adult nephrotic syndrome and lower PON1 might contribute to atherosclerosis (14). Mohammed Hashemi et al evaluated paraoxonase in children with nephrotic syndrome in 2013 (11). Ece et al studied paraoxonase, total antioxidant response and peroxide level in children with nephrotic syndrome. They also reported oxidant antioxidant imbalance in active phase of nephrotic syndrome (16). In line with these authors we have found in our study that the PON1 levels are highly decreased in nephrotic syndrome patients ( $p < 0.001$ ). Therefore determination of paraoxonase activity might be a biomarker for atherosclerosis and cardiovascular risk in nephrotic syndrome patients.

Between SPON and BPON, SPON activity was higher than the BPON activity in both cases and controls. But the SPON activities of cases were less than even the BPON activity of the controls. An ROC plot was made to compare the sensitivity and specificity of BPON and SPON in nephrotic syndrome cases. BPON exhibited slight increase in area under the ROC curve when compared to SPON. Bindu et al in 2011 studied the sensitivity and specificity of basal PON1 and salt stimulated PON 1 in liver diseases by ROC curve, in which they found a higher sensitivity and specificity for basal PON1 activity to assess the liver function (15).

The levels of HDL cholesterol were higher in controls, while all the other lipid profile parameters were lower in controls when compared to the cases. Atherogenic Index was significantly higher in cases than the controls. Also there was a significant negative correlation between atherogenic index and PON1 levels in nephrotic syndrome cases. That is, as PON1 levels decreases, the Atherogenic index increases. Therefore PON1 can be used as a measure of Atherogenic risk in nephrotic syndrome patients.

Paraoxonase, is a calcium dependent enzyme found in liver, intestine, kidney, serum etc. It has the ability to prevent LDL oxidation, destroy modified phospholipids and prevent accumulation of oxidized lipids in lipoproteins thereby performing anti-inflammatory and anti-atherogenic functions (17). Animal studies prove that paraoxonase 1 is involved in the attenuation of atherosclerosis. Paraoxonase1 was found inversely correlated to atherogenic index in a study conducted in healthy children by Sumegova (18). In their study there was no association between PON1 and other lipid profile parameters.

Lower PON1 activity can be due to increased oxidative stress in nephrotic syndrome. Also dyslipidemia in nephrotic syndrome causes increased LDL and decreased HDL levels. High LDL levels, in the absence of ample amount of HDL associated PON1, gets oxidized leading to increased ox-LDL. This can lead to increased risk of cardiovascular risk in nephrotic syndrome. Therefore determination of PON1 along with other cardiovascular markers may be performed in nephrotic syndrome patients to prevent future risk of atherosclerosis.

## CONCLUSIONS

The study reveals an increased risk of cardiovascular disease in nephrotic syndrome patients. Hence measuring PON1 and Atherogenic index during the initial stages of nephrotic syndrome may help in predicting the extent of the associated cardiovascular risk.

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## APPENDICES

**Table 1: Paraoxonase Levels and Lipid Profile in Nephrotic Syndrome Patients and Controls**

Sl. No	Parameter	Cases (n=40) (Mean±SD*)	Controls (n=40) (Mean±SD*)	P Value
1	PON 1 basal (IU/ml)	66.11±16.99	82.16±13.54	<0.001**
2	PON 1 salt stimulated (IU/ml)	75.03±17.32	90.42±13.73	<0.001**
3	Total Cholesterol (mg/dl)	286.78±144.8	161.56±51.23	<0.001**
4	HDL Cholesterol (mg/dl)	47.63±18.04	54.92±12.12	0.027**
5	LDL Cholesterol (mg/dl)	199.18±128.8	83.15±49.77	<0.001**
6	VLDL Cholesterol (mg/dl)	23.49±10.12	45.02±27.12	<0.001**
7	Triglycerides (mg/dl)	225.8±135.52	117.4±50.60	<0.001**
8	Atherogenic Index	0.39±0.19	0.24±0.15	<0.001**

\*SD – Standard Deviation, \*\*p value - <0.05 indicates that the results are highly significant

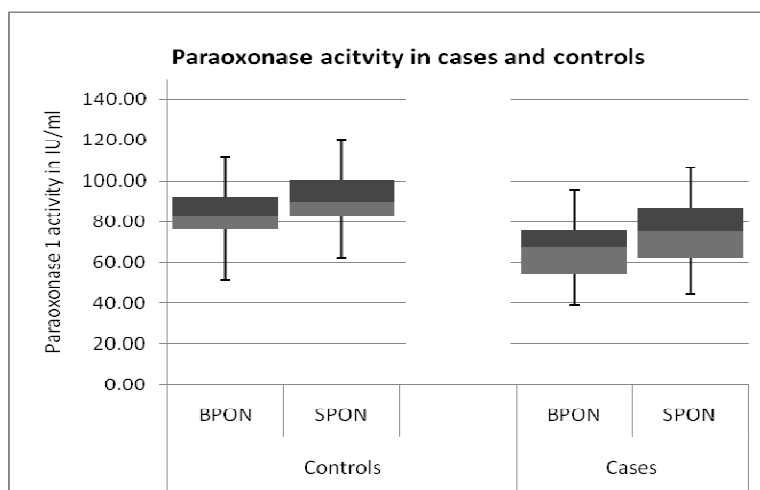


Figure 1: PON 1 Activity in Cases and Controls

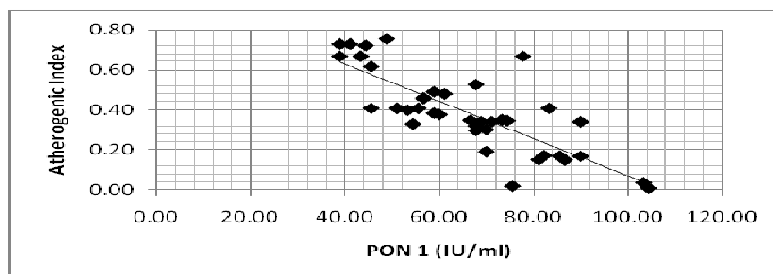


Figure 2: Correlation between PON1 and Atherogenic Index

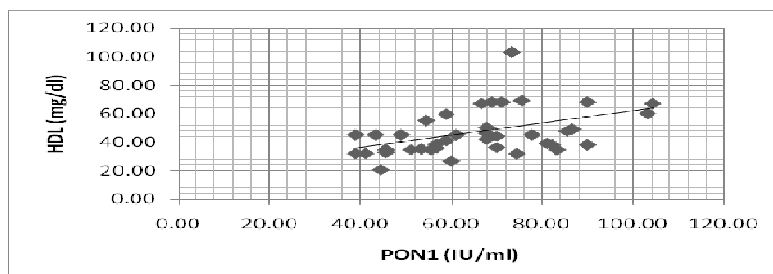
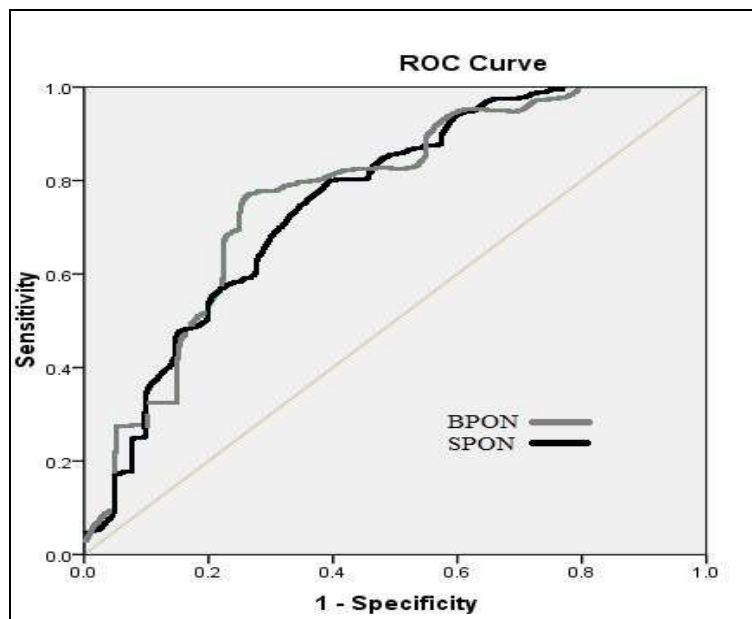


Figure 3: Correlation between PON1 and HDL Cholesterol



**Figure 4: ROC Curve**

#### Figure Legends

**Figure 1:** PON 1 activity in cases and controls

**Legend:** Box and whisker plot – serum basal and salt stimulated Paraoxonase 1 activity. The boxes extend between 25<sup>th</sup> and 75<sup>th</sup> percentile. The whiskers indicate the minimum and maximum value for the two groups.

**Figure 2:** Correlation between PON1 and Atherogenic Index

**Legend:** The graph shows a strong negative correlation between Atherogenic Index and PON 1. Pearson correlation coefficient,  $r = 0.798$

**Figure 3:** Correlation between PON1 and HDL cholesterol

The graph shows an intermediate positive correlation between HDL cholesterol and PON 1. Pearson correlation coefficient,  $r = 0.479$

**Figure 4:** ROC curve

**Legend:** Receiver Operating Characteristic curves. The graph shows that both BPON and SPON has high sensitivity and specificity for assessing the cardiovascular risk in nephrotic syndrome. The AUC is 0.770 for BPON and 0.758 for SPON.